

Marx, Irene

From: Weber, Jon
Sent: Tuesday, July 15, 2003 5:16 PM
To: Marx, Irene
Subject: 11-056833

* NOTICES *

Japan Patent Office is not responsible for any damages caused by the use of this translation.

1. This document has been translated by computer. So the translation may not reflect the original precisely.
2. **** shows the word which can not be translated.
3. In the drawings, any words are not translated.

DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[The technical field to which invention belongs] this invention relates to the method of it being efficient and manufacturing a polyamine constituent in large quantities, from yeast-fungus object contents. After digesting yeast-fungus object contents by the nuclease or hydrolyzing with alkali in detail, it is related with the manufacturing method of the polyamine constituent which collects polyamine. It faces manufacturing a polyamine constituent from yeast-fungus object contents, and the recovery of polyamine can be raised by applying the method of this invention.

[0002]

[Description of the Prior Art] Polyamine is the general term of the aliphatic hydrocarbon of the shape of a straight chain with two or more first-class amino groups, and can mention a putrescine, a spermidine, and a spermine as typical polyamine. As a physiological function of polyamine, it is (1) A proliferation-of-cells operation and (2) A cell differentiation promotion operation and (3) An immunity indispensable factor and (4) An anti-allergy operation and (5) A protein synthesis promotion operation and (6) Stabilization of the structure by the interaction with a nucleic acid, and (7) The enzyme activity regulation operation etc. is known. Recently, many reports are made about the effect that the polyamine which carried out the ingestion promotes multiplication and specialization of an alimentary canal membrane cell. (O. Peulen et al. and Arch.) Physiol. Biochem., vol.106, pp.46-55, 1998; W.P. Deloyer et al., Arch. Physiol. Biochem., vol.104, pp.163-172, 1996; M. Kaouass et al., Dig. Dis. Sci., vol.41, pp.1434-1444, 1996; E. Harada et al., Comp. Biochem. Physiol., vol.109A, pp.667-673, 1994; G. Capano et al. and J. Pediatr. Gastroenterol. Nutr., vol.19, pp.34-42, and 1994; G. E. Wild et al., Biol. Neonate, vol.63, pp.246-257, 1993; Buts J.-P. et al. and Digestive Diseases and Science, vol.38, p.1091, 1993; Dufour, C. et al., Gastroenterology, vol.95, p.112, 1988.

[0003] In these reports, it is shown clearly that it is investigated about the physiology effect of a spermidine or a spermine, and the spermine has the high alimentary canal mature promotion operation rather than the spermidine. Furthermore, it is also reported that it is reported that the polyamine which carried out the ingestion is incorporated inside of the body promptly, and is used in an organization, and a spermidine and the spermine are promptly absorbed rather than a putrescine (Bardocz, S. et al., J. Nutr. Biochem., vol.4, p.66, 1993). And the thing for which a spermine and a spermidine are added as an example which used polyamine for food. polyamine combination nutrition constituent which promotes proteinic absorption and makes good growth and good health condition hold by blending konnyaku (publication-number 6-No. 38690 official report) and

polyamine which do not have a bad influence even if it reduces a smell peculiar to konnyaku and cooks with other food (JP,6-305956,A) etc. -- it is proposed Moreover, it considers as the example which used polyamine as medical supplies, and the method and the constituent for ingestion for gastric-acid secretion prevention (Provisional-Publication-No. 58 No. -131914 official report) which prevent gastric-acid secretion, the immunostimulator (JP,59-98015,A and JP,2-223514,A), etc. are proposed.

[0004] By the way, it is known that there are many amounts of polyamine contained in food at fermented foods, such as meat, a cheese head, and bean paste, and it is few with milk or vegetables (Bardocz, S.et al., J.Nutr.Biochem, vol.4, p.66, the collection of the 12th time research presentation meeting lecture summaries of 1993; polyamine study group, p.4, 1995). Therefore, there are very few amounts of polyamine contained in nutrition constituents, such as modified milk powder for sucklings which uses cow's milk as the main raw material. It is reported that comparatively a lot of polyamine is contained in human milk on the other hand. (a Japanese child nutrition digestive organ disease society magazine, vol.9, no.2, pp.115-121, 1995) It can be called desirable thing from a physiological viewpoint to strengthen polyamine to a nutrition constituent with few polyamine contents. In addition, although proteolysis milk is introduced by Buts and others as a nutrition constituent with a high polyamine content (Buts, J.P.et al., J.Pediatr.Gastroenterol.Nutr., vol.21, p.44, 1995), the polyamine contained in this proteolysis milk is polyamine originating in the crude enzyme used for proteolysis. It is not a thing aiming at strengthening of polyamine.

[0005] Moreover, the nutrition constituent which blended the polyamine manufactured by the manufacturing method and this method of polyamine from a yeast-fungus object is proposed. (JP,10-52291,A) . By this method, polyamine without the nasty smell taste can be manufactured by processing a yeast-fungus object under acid conditions. However, under acid conditions, since a part of polyamine precipitated with the polymeric material, all the polyamine contained in a yeast biomass was unrecoverable. Moreover, since a part of polyamine was combined with the polymeric material in the living body, no polyamine was unrecoverable even if it performed fractionation processing as it was.

[0006]

[Problem(s) to be Solved by the Invention] When this invention persons came research in piles wholeheartedly, by digesting yeast-fungus object contents by the nuclease, or understanding an added water part with alkali, they find out that a polyamine constituent is recoverable with efficient high recovery and came to complete this invention to develop the method of it being efficient and manufacturing a polyamine constituent in large quantities. Therefore, this invention makes it a technical problem to offer the method of manufacturing a polyamine constituent from yeast-fungus object contents with efficient high recovery.

[0007]

[Means for Solving the Problem] In this invention, polyamine is collected and a polyamine constituent is manufactured, after facing manufacturing a polyamine constituent, using yeast-fungus object contents as a raw material, and digesting these yeast-fungus object contents by the nuclease or hydrolyzing with alkali. It can crush physically, and can extract or can extract by hot water, or autolysis of baker's yeast, wine yeast, beer yeast, the torula yeast, etc. can be carried out, they can be extracted, and the yeast-fungus object contents which can be used as a raw material by this invention can prepare them. What is necessary is to crush a yeast-fungus object by the high-pressure homogenizer, ultrasonic crush, etc., and just to extract yeast-fungus object contents, for example, as a method of crushing a yeast-fungus object physically and extracting yeast-fungus object contents. In addition, the high-pressure homogenizer to be used has the capacity which can do damage to a cell wall or a cell membrane, and causes exchange of the liquid in a biomass, and the liquid outside a biomass, what can take out a two or more 700 kgf/cm pressure is desirable, and such a high-pressure homogenizer is used for it. What is necessary is just to crush a yeast-fungus object by the pressure of 700 - 1,400 kgf/cm². Such a high-pressure homogenizer is manufactured in RANI, a gaulin company, and NIPPON SEIKI CO., LTD. Moreover, what can destroy a cell mechanically is desirable, and uses such an ultrasonic crusher, and the ultrasonic crusher to be used is 10-90kHz. What is necessary is just to crush biomass suspension in several minutes and several steps from dozens of seconds. Such an ultrasonic crusher is Branson. It is manufactured at a shrine, Ultrasonic, and Rayton.

[0008] As a method of extracting yeast-fungus object contents from a yeast-fungus object by hot water Yeast-fungus object concentration desirably 5 to 25% for example, to 10 - 20% of biomass suspension salt

concentration becomes 4 - 8% desirably 1 to 10% -- as -- salt -- adding -- pH -- 4-8 -- desirable -- pH five to 7 temperature -- 90-100 °C -- what is necessary is to warm desirably by 95 - 100 °C for 3 to 5 hours for 1 to 5 hours, and just to extract yeast-fungus object contents What is necessary is to add autolysis accelerators, such as salt used, for example as a method of carrying out autolysis of the yeast-fungus object, and extracting yeast-fungus object contents in case a yeast extract is manufactured, fatty acid ester, an organic acid, and an organic solvent, and just to promote the autolysis of a yeast-fungus object (JP,54-13496,B, JP,55-34096,A, Provisional-Publication-No. 59 No. -109152 official report). . Moreover, what is necessary is to give mechanical stimulus, such as hydrostatic-pressure processing of extra-high voltage, ultrasonication, and high-pressure homogenizer processing, and just to promote the autolysis of a yeast-fungus object. (JP,2-255059,A, JP,50-25539,B) . In addition, by holding a yeast-fungus object at the temperature of 37-55 degrees C, autolysis of the protein and RNA in a biomass is carried out, and they are decomposed into amino acid or a 3'-nucleotide and 5'-nucleotide, respectively.

[0009] Moreover, a commercial yeast extract and a commercial yeast ribonucleic acid can also be used as yeast-fungus object contents. As a commercial yeast extract etc., for example RN, RN7, radiographic, RN7-P, RB2-P and radiographic-P (above, made in SAPPORO BREWERIES Agency) The I strike S, the I strike N, the I strike PIG, the super I strike R-1, the super I strike powder A-001, the super I strike R-7, dryness beer yeast Y2A, beer yeast extract < Ebios > P2G (above, the Asahi Breweries food company make) Dryness beer yeast (the Asahi Breweries chemical company make) Yeast-extract C, yeast-extract W, yeast-extract L and yeast-extract H (above, Kyowa Hakko Kogyo Co., Ltd. make) Yeast extract emic -- YG (Tanabe Seiyaku Co., Ltd. make) Dryness beer yeast BY-S, debittering yeast BY-G, and yeast ribonucleic acid (above, KIRIN BREWERY CO., LTD. make) etc. -- it can mention

[0010] As a method of digesting yeast-fungus object contents by the nuclease by this invention, a nuclease is added to the solution containing yeast-fungus object contents, and it is at pH 3-10 and 10-70 degrees C, for example. What is necessary is just to process for 0.1 to 24 hours. as the nuclease to be used -- DNase I, a nuclease, a nuclease P1, a nuclease S1, the phosphodiesterase I, RNase A, RNase B, RNase T1, and RNase T2 RNase U2 etc. -- you may process with the organization of vegetation or an animal which may process as it is by the nuclease of commercial elegance, and has nuclease activity that what is necessary is just what has the property which decomposes a nucleic acid, a microorganism biomass, microbial cultivation liquid, etc. Furthermore, it is also possible to process by the nuclease which the yeast-fungus object contents itself to be used have.

[0011] As a method of understanding yeast-fungus object contents an added water part with alkali by this invention, it is in the solution containing yeast-fungus object contents, for example. Alkali is added so that it may be set to 0.1-5N, and it is at 20-100 degrees C. What is necessary is just to process for 0.1 to 24 hours. A sodium hydroxide, a potassium hydroxide, etc. can be mentioned as alkali to be used. Thus, after digesting yeast-fungus object contents by the nuclease or hydrolyzing with alkali, a polyamine constituent can be manufactured by collecting polyamine.

[0012]

[Embodiments of the Invention] Nuclease digestion of these yeast-fungus object contents is carried out by making into yeast-fungus object contents the yeast-fungus object contents prepared from baker's yeast, beer yeast, torula yeast, etc. or a commercial yeast extract, and a yeast ribonucleic acid, or it understands an added water part with alkali. And a polyamine constituent can be manufactured by collecting polyamine. Thus, about the obtained polyamine constituent, it can be used with a solution, and spray drying, freeze drying, etc. can be performed and it can also be used as powder. Furthermore, after performing desalting and refining, it can also be used as powder with a solution. In addition, when using a commercial yeast extract and a commercial yeast ribonucleic acid as yeast-fungus object contents, the content ratio of the spermidine and spermine which are high polyamine of the physiology effect from an acid beforehand about a yeast extract or a yeast ribonucleic acid 1 time or by processing several times, discarding a supernatant liquid, collecting sedimentation, and using this sedimentation as yeast-fungus object contents can be raised, and polyamine composition can also be adjusted. Moreover, after digesting yeast-fungus object contents by the nuclease or hydrolyzing with alkali, by carrying out deproteinization, a polyamine content can be raised and the load in the case of refining further can also be mitigated. As a method of carrying out deproteinization from a polyamine solution, acid treatment, a

salting-out, protease digestion, etc. can be mentioned.

[0013] What is necessary is to add an acid, to make pH or less into two, to remove the sedimentation which left it for 2 to 6 hours, and generated, and just to collect supernatant liquids as a method of carrying out deproteinization by acid treatment, for example, after cooling a polyamine solution at 10 degrees C or less. As an acid to be used, inorganic acids and organic acids, such as a sulfuric acid, a hydrochloric acid, an acetic acid, a phosphoric acid, trichloroacetic acid, perchloric acid, a sulfosalicylic acid, and a formic acid, can be mentioned. What is necessary is to add salts, such as an ammonium sulfate, to a polyamine solution, and to cool at 5 degrees C or less that what is necessary is to add salts, such as ferric chloride, to a polyamine solution, and to heat as a method of carrying out deproteinization by the salting-out for several hours, for example, and to make protein and other impurities condense, and for processing of centrifugal separation etc. to remove, and just to collect supernatant liquids, and to make protein condense, and for processing of centrifugal separation etc. to remove, and just to collect supernatant liquids.

[0014] As a method of carrying out deproteinization by protease digestion, a protease is added to a polyamine solution and it is at pH 1-10 and 10-70 degrees C, for example. What is necessary is just to process for 0.1 to 24 hours. As a protease to be used, they are a protease originating in animals and plants, such as a trypsin and a papain, or an Aspergillus (Aspergillus). You may process with the organization of vegetation or an animal which may process as it is by the protease of commercial elegance, and has protease activity, a microorganism biomass, microbial cultivation liquid, etc. that the protease which microorganisms, such as ****, rhizopus (Rhizopus) ****, and bacillus (Bacillus) ****, produce should just be what has the property which disassembles protein. Moreover, you may perform desalting and refining for a polyamine solution with meanses, such as ion-exchange-resin processing, film fractionation, and an electrodialysis, if needed. In addition, the polyamine constituent of a high grade can be obtained more by combining these meanses suitably.

[0015] As the method of ion-exchange-resin processing, a polyamine solution is dipped in the column filled up with ion exchange resin, for example, and polyamine and impurity, such as amino acid, a peptide, protein, and a saccharide, are separated. As ion exchange resin to be used, an ion exchange group can all also use a cation exchange resin or an anion exchange resin that what is necessary is just a sulfonic group, a sulfo propyl group, a phosphoric-acid machine, a carboxyl methyl group, an aminoethyl machine, a diethylamino machine, the 4th class aminoethyl machine, the 4th class ammonium, etc. In addition, when a cation exchange resin is used, since a cation exchange resin is adsorbed, after polyamine fully separates a non-adsorbate, with salting-in liquid, such as acidic solutions, such as a sulfuric acid and a hydrochloric acid, and a sodium chloride, it is eluted and should just collect polyamine. Moreover, when an anion exchange resin is used, since an anion exchange resin is not adsorbed, polyamine should just collect the non-adsorbing fractions containing polyamine.

[0016] As the method of film fractionation, a cut off molecular weight by a cellulose system, a cellulose acetate system, a polysulfone system, a polyamide system, the poly acrylic nitril system, the polytetrafluoroethylene system, the polyester system, the polypropylene system, etc., for example 1,000-100,000 What is necessary is to perform UF of a polyamine solution using the ultrafiltration (UF) film of the range, and just to collect the transparency liquid containing polyamine. Moreover, nano philharmonic tray SHON of 30 - 80% of salt rejection (NF) You may desalt by performing NF of a polyamine solution using a film. What is necessary is to supply a polyamine solution and brine by turns between each film divided by the cation exchange membrane and the anion exchange membrane as the method of an electrodialysis, for example, and just to perform an electrodialysis. In addition, the conditions of an electrodialysis are initial-current density. 0.5 - 15 A/dm² Voltage A 0.1 - 1.5V/tub is desirable. Thus, the obtained polyamine constituent can be further used as fortification food or an additive of common food as medical-application nutrition constituents, such as physisic and an enteral hyperalimentation drug, and nutrition constituents for infants, such as milk powder for puericulture, and a baby food. Next, an example and the example of comparison are shown and this invention is explained in more detail. In addition, analysis of the polyamine content in an example and the example of comparison was performed according to the method (a Japanese child-nutrition digestive organ disease society magazine, vol.9, pp.115-121, 1995) of upper parts of rivers.

[0017]

[Example 1] Torula yeast (*Candida utilis*) The biomass was used and the polyamine constituent was manufactured by digesting yeast-fungus object contents by the nuclease. Torula yeast (*Candida utilis*)

Inoculation was carried out to the molasses culture medium, and the aeration spinner culture was performed at 30 degrees C for 48 hours. At long-intervals heart separation (5,000g) of the culture medium was carried out at 4 degrees C after cultivation for 30 minutes, and the harvest of the yeast-fungus object was carried out. After cold water washes the yeast-fungus object which carried out the harvest, yeast-fungus object concentration prepares the biomass suspension which is 15%, and salt concentration At 4.8%, pH 6.0, and 95 degrees C Yeast-fungus object contents were extracted on the conditions of 3.5 hours. Ferric chloride is added to the solution of these yeast-fungus object contents, and it is pH. After adjust to 5.0, heating for 90 minutes at 90 degrees C, making protein and other impurity condense, filtering with diatomaceous earth and collecting supernatant liquids, this supernatant liquid is cooled to 5 degrees C, a hydrochloric acid is added, and it is pH. It adjusted to 1.5, it was left for 4 hours, polyamine was settled, and it collected. After suspending this sedimentation in water, a sodium hydroxide is added 30% and it is pH. It adjusted and dissolved in 6.0, and nuclease A(Kanto chemistry company make) 1mg/ml was added, it digested at 25 degrees C for 15 hours, and the polyamine solution was obtained. After dipping this polyamine solution in the column filled up with the cation exchange resin (Dowex 50WX8 (H+ type)) and making polyamine stick to a cation exchange resin, 0.7M brine was dipped in the column, the resin was washed enough, and the polyamine which removed the impurity and adsorbed it was eluted with 6-N hydrochloric acid. And after adding the sodium-hydroxide solution to this eluate 30% and neutralizing, it electro-dialyzed, and it desalted, and it freeze-dried and the polyamine constituent was manufactured. Thus, it hits 1kg (wet weight) of yeast-fungus objects, and is polyamine. Polyamine constituent containing 465mg 698mg was obtained. This is a conventional method. (example 1 of comparison) Compared with the yield of the polyamine constituent to twist, it has increased 3 times. Moreover, polyamine It is the total quantity of a spermidine and a spermine among 465mg. The rate of the spermidine which is 446mg and is occupied to the whole polyamine, and a spermine was increasing by 96%.

[0018] . .

[Example 2] Wine yeast (*Saccharomyces cerevisiae*) The biomass was used and the polyamine constituent was manufactured by digesting yeast-fungus object contents by the nuclease. Wine yeast (*Saccharomyces cerevisiae*) A biomass is suspended in water, the biomass suspension whose yeast-fungus object concentration is 10% is prepared, and it is a high-pressure homogenizer (TYPE10.51VH, product made from RANNIE). It was used, physical spallation was performed by pressure 1,000 kgf/cm², and yeast-fungus object contents were extracted. the solution of these yeast-fungus object contents -- 30% sodium hydroxide -- adding -- pH 8.0 -- adjusting -- RNase A (the Kanto chemistry company make) and a trypsin (made in BERINGA Mannheim) -- ml was added in 1mg /, respectively, it digested at 37 degrees C for 18 hours, and the polyamine solution was obtained It is a PLCC cellulose membrane (cut-off-molecular-weight 5,000, Millipore Corp. make) about this polyamine solution. After having used it, performing UF and removing polymeric materials which remain, such as an enzyme and non-decomposed protein, UF transparency liquid was dipped in the column filled up with the anion exchange resin (Dowex 1X8 (Cl- type)), the amino acid with which are contaminated was made to stick to an anion exchange resin, and was removed, and non-adsorbing fractions were collected. And this solution was freeze-dried and the polyamine constituent was manufactured. Thus, polyamine constituent which hits 1kg (wet weight) of yeast-fungus objects, and contains polyamine 87mg 183mg was obtained. This is a conventional method. (example 2 of comparison) It compares with the yield of the polyamine constituent to twist. It has increased 2.1 times. Moreover, the rate of the spermidine which the total quantity of a spermidine and a spermine is 82.7mg in polyamine 87mg, and is occupied to the whole polyamine, and a spermine was increasing by 95%.

[0019]

[Example 3] The yeast ribonucleic acid (KIRIN BREWERY CO., LTD. make) was used as yeast-fungus object contents, and the polyamine constituent was manufactured by understanding yeast-fungus object contents an added water part with alkali. It dissolved in 0.3-N potassium hydroxide so that yeast ribonucleic-acid concentration might become 5%, and it understood an added water part at 37 degrees C for 18 hours, and the polyamine solution was obtained. After dipping this polyamine solution in the column filled up with the cation exchange resin (Dowex 50WX8 (H+ type)) and making polyamine stick to a cation exchange resin, 0.5M brine was dipped in the column, the resin was washed enough, and the polyamine which removed the impurity and adsorbed it was eluted with 6-N hydrochloric acid. And after adding the sodium-hydroxide solution to this

eluate and neutralizing, it electrodialed, and it desalted, and it freeze-dried and the polyamine constituent was manufactured. Thus, per 1kg of yeast ribonucleic acids, polyamine Polyamine constituent containing 1,460mg 1,750mg was obtained. This is a conventional method. (example 3 of comparison) It compares with the yield of the polyamine constituent to twist. It has increased 3.2 times. Moreover, polyamine It is the total quantity of a spermidine and a spermine among 1,460mg. The rate of the spermidine which is 1,431mg and is occupied to the whole polyamine, and a spermine was increasing by 98%.

[0020]

[Example 4] Baker's yeast (*Saccharomyces cerevisiae*) The biomass was used and the polyamine constituent was manufactured by carrying out autolysis of the yeast-fungus object contents. Baker's yeast (*Saccharomyces cerevisiae*) 1kg of this biomass suspension after suspending a biomass in water and preparing the biomass suspension whose yeast-fungus object concentration is 20% It moved to the flask of 2 l **, and 8g of lactic acids was added, autolysis was carried out at 45 degrees C for 24 hours, by warming to 90 degrees C after that, and holding for 10 minutes, autolysis was stopped and the polyamine solution was obtained. After dipping this polyamine solution in the column filled up with the cation exchange resin (Dowex 50WX8 (H⁺ type)) and making polyamine stick to a cation exchange resin, 0.6M brine was dipped in the column, the resin was washed enough, and the polyamine which removed the impurity and adsorbed it was eluted with 6-N hydrochloric acid. And after adding the sodium-hydroxide solution to this eluate and neutralizing, it electrodialed, spray drying was desalted and carried out, and the polyamine constituent was manufactured. Thus, polyamine constituent which hits 1kg (wet weight) of yeast-fungus objects, and contains polyamine 95mg 314mg was obtained. Moreover, the total quantity of a spermidine and a spermine was 88.4mg in polyamine 95mg.

[0021]

[The example 1 of comparison] Torula yeast (*Candida utilis*) The biomass was used and the polyamine constituent was manufactured. Torula yeast (*Candida utilis*) Inoculation was carried out to the molasses culture medium, and the aeration spinner culture was performed at 30 degrees C for 48 hours. At-long-intervals heart separation (5,000g) of the culture medium was carried out at 4 degrees C after cultivation for 30 minutes, and the harvest of the yeast-fungus object was carried out. After cold water washes the yeast-fungus object which carried out the harvest, yeast-fungus object concentration prepares the biomass suspension which is 15%, and salt concentration At 4.8%, pH 6.0, and 95 degrees C Yeast-fungus object contents were extracted on the conditions of 3.5 hours. Ferric chloride is added to the solution of these yeast-fungus object contents, and it is pH. After adjust to 5.0, heating for 90 minutes at 90 degrees C, making protein and other impurity condense, carrying out suction filtration using cerite (made in John-Mann Bill) and collecting supernatant liquids, this supernatant liquid is cooled to 5 degrees C, a hydrochloric acid is added, and it is pH. It adjusted to 1.5, it was left for 4 hours, polyamine was settled, and it collected. After suspending this sedimentation in water, a sodium hydroxide is added 30% and it is pH. It adjusted and dissolved in 6.0 and the polyamine solution was obtained. After dipping this polyamine solution in the column filled up with the cation exchange resin (Dowex 50WX8 (H⁺ type)) and making polyamine stick to a cation exchange resin, 0.7M brine was dipped in the column, the resin was washed enough, and the polyamine which removed the impurity and adsorbed it was eluted with 6-N hydrochloric acid. And after adding the sodium-hydroxide solution to this eluate 30% and neutralizing, it electrodialed, and it desalted, and it freeze-dried and the polyamine constituent was manufactured. Thus, it hits 1kg (wet weight) of yeast-fungus objects, and is polyamine. Polyamine constituent containing 155mg 500mg was obtained. moreover, polyamine the inside of 155mg -- the total quantity of a spermidine and a spermine 142.6mg (92%) it was .

[0022]

[The example 2 of comparison] Wine yeast (*Saccharomyces cerevisiae*) The biomass was used and the polyamine constituent was manufactured. Wine yeast (*Saccharomyces cerevisiae*) A biomass is suspended in water, the biomass suspension whose yeast-fungus object concentration is 10% is prepared, and it is a high-pressure homogenizer (TYPE10.51VH, product made from RANNIE). It was used, physical spallation was performed by pressure 1,000 kgf/cm², and yeast-fungus object contents were extracted. It is a PLCC cellulose membrane (cut-off-molecular-weight 5,000, Millipore Corp. make) about the solution of these yeast-fungus object contents. After having used it, performing UF and removing a polymeric material, UF transparency liquid was dipped in the column filled up with the anion exchange resin (Dowex 1X8 (Cl⁻ type)), the amino acid with

which are contaminated was made to stick to an anion exchange resin, and was removed, and non-adsorbing fractions were collected. And this solution was freeze-dried and the polyamine constituent was manufactured. Thus, polyamine constituent which hits 1kg (wet weight) of yeast-fungus objects, and contains polyamine 42mg 220mg was obtained. moreover, the inside of polyamine 42mg -- the total quantity of a spermidine and a spermine -- 37.8mg (90%) it was .

[0023]

[The example 3 of comparison] The yeast ribonucleic acid (KIRIN BREWERY CO., LTD. make) was used as yeast-fungus object contents, and the polyamine constituent was manufactured. It dissolved in water so that yeast ribonucleic-acid concentration might become 5%, and the polyamine solution was obtained. After dipping this polyamine solution in the column filled up with the cation exchange resin (Dowex 50WX8 (H+ type)) and making polyamine stick to a cation exchange resin, 0.5M brine was dipped in the column, the resin was washed enough, and the polyamine which removed the impurity and adsorbed it was eluted with 6-N hydrochloric acid. And after adding the sodium-hydroxide solution to this eluate and neutralizing, it electrodialed, and it desalted, and it freeze-dried and the polyamine constituent was manufactured. Thus, per 1kg of yeast ribonucleic acids, polyamine Polyamine constituent containing 460mg 550mg was obtained. moreover, polyamine the inside of 460mg -- the total quantity of a spermidine and a spermine 437mg (95%) it was .

[0024]

[Effect of the Invention] It faces carrying out extensive manufacture of the polyamine constituent from yeast-fungus object contents, and the recovery of polyamine can be raised by applying the method of this invention. Moreover, the rate for which the spermidine said to be effective among polyamine and a spermine account can also be raised.

[Translation done.]

Jon P. Weber, Ph.D.

AU 1651
703-308-4015
CM1-11B07
Mail-11B01